bio-protocol Preprint

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Plant material and growth conditions

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Updated date: Jul 11, 2020

An abbreviated version of this protocol was published in eLIFE in Jul 2015

Seasonal shift in timing of vernalization as an adaptation to extreme winter

DOI: 10.7554/eLife.06620

Detailed protocol

Plant Material and Growth Conditions

1) ~10 seeds from each genotype (Col *FRI* ^{Sf2}, Edi-0, Lov-1, Ull2-5 and Var2-6) were sown directly into each well containing moistened compost in 24 well plastic trays and stratified for 3 days at 4 ^{o}C in a walk-in room with an 8 hour light, 16 hour dark cycle (~30 µmol m^{-2} s^{-1}).

2) Seedlings were pre-grown for 7 days in a walk-in controlled environment room set to 22 ^{o}C (± 2 ^{o}C) with a 16 hour light, 8 hour dark cycle (100 µmol m^{-2} s^{-1}).

3) Vernalization was carried out for either 0, 4, 6 or 12 weeks under an 8 hour light, 16 hour dark cycle (~30 µmol m^{-2} s^{-1}). Conditions were monitored throughout and recorded as being consistently close to both the target temperature ± 1.5 ^{o}C and within a relative humidity range of 70 % ± 10 %.

Sanyo cabinets (Sanyo MLR-351H) were used to vernalize plants at 14 ^{o}C, 12 ^{o}C, 10 ^{o}C and 8 ^{o}C.
5 ^{o}C vernalization was carried out in a walk-in room at John Innes Centre.
2^{o}C treatment was achieved by placing plants in a Liebherr KP3120 fridge containing a timed LED light strip custom-made by the JIC maintenance department.
Pre-growth conditions were replicated before plants were vernalized at 0^{o}C using Johnson Control cabinets located at Gregor Mendel Institute, Vienna, Austria.

4) Following vernalization, plants were transferred to random locations in a walk-in controlled environment room that was set to provide 16 hours light (100 µmol m^{-2} s^{-1}) and 8 hours dark cycles at 22 ^{o}C (± 2 ^{o}C). After four days, seedlings were thinned out to one plant per well.

5) Flowering time was scored for a minimum of 10, and a maximum of 12 plants, per genotype, per vernalization temperature, per vernalization treatment length.

6) Days to flower were recorded as the number of days growth in permissive conditions until floral buds became visible by eye.

7) This experiment was repeated 3 times to facilitate statistical analysis.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Duncan, S. (2020). Plant material and growth conditions. Bio-protocol Preprint. bio-protocol.org/prep384.
2. Duncan, S., Holm, S., Questa, J., Irwin, J., Grant, A. and Dean, C.(2015). Seasonal shift in timing of vernalization as an adaptation to extreme winter. eLIFE. DOI: 10.7554/eLife.06620